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A comparison of the pharmacokinetics of perfluorobutanesulfonate (PFBS) in rats, monkeys, and humans

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ABSTRACT

Materials derived from perfluorobutanesulfonyl fluoride (PBSF, $C_4F_9SO_2F$) have been introduced as replacements for eight-carbon homolog products that were manufactured from perfluorooctanesulfonyl fluoride (POSF, $C_8F_{17}SO_2F$). Perfluorobutanesulfonate (PFBS, $C_4F_9SO_3^-$) is a surfactant and potential degradation product of PBSF-derived materials. The purpose of this series of studies was to evaluate the pharmacokinetics of PFBS in rats, monkeys, and humans, thereby providing critical information for human health risk assessment. Studies included: (1) intravenous (i.v.) elimination studies in rats and monkeys; (2) oral uptake and elimination studies in rats; and (3) human serum PFBS elimination in a group of workers with occupational exposure to potassium PFBS (K^+PFBS). PFBS concentrations were determined in serum (all species), liver (rats), urine (all species), and feces (rats). In rats, the mean terminal serum PFBS elimination half-lives, after i.v. administration of 30 mg/kg PFBS, were: males 4.51 ± 2.22 h (standard error) and females 3.96 ± 0.21 h. In monkeys, the mean terminal serum PFBS elimination half-lives, after i.v. administration of 10 mg/kg PFBS, were: males 95.2 ± 27.1 h and females 83.2 ± 41.9 h. Although terminal serum half-lives in male and female rats were similar, without statistical significance, clearance (CL) was significantly greater in female rats (469 ± 40 mL/h) than male rats (119 ± 34 mL/h) with the area under the curve (AUC) significantly larger in male rats (294 ± 77 μg·h/mL) than female rats (65 ± 5 μg·h/mL). These differences were not observed in male and female monkeys. Volume of distribution estimates suggested distribution was primarily extracellular in both rats and monkeys, regardless of sex, and urine appeared to be a major route of elimination. Among 6 human subjects (5 male, 1 female) followed up to 180 days, the geometric mean serum elimination half-life for PFBS was 25.8 days (95% confidence interval 16.6–40.2). Urine was observed to be a pathway of elimination in the human. Although species-specific differences exist, these findings demonstrate that PFBS is eliminated at a greater rate from human serum than the higher chain homologs of perfluorooctanesulfonate (PFOS) and perfluorohexanesulfonate (PFHxS). Thus, compared to PFOS and PFHxS, PFBS has a much lower potential for accumulation in human serum after repeated occupational, non-occupational (e.g., consumer), or environmental exposures.

1. Introduction

Materials derived from perfluorobutanesulfonyl fluoride (PBSF, $C_4F_9SO_2F$) have been introduced by the 3M Company as replacements for its eight-carbon homolog products that were manufactured from perfluorooctanesulfonyl fluoride (POSF, $C_8F_{17}SO_2F$). 3M phased out of manufacturing POSF-based materials after a metabolite and environmental degradation product, perfluorooctanesulfonate (PFOS, $C_8F_{17}SO_3^-$), was found to be widespread in human populations and wildlife (Butenhoff et al., 2006; Houde et al., 2006). Hydrolysis of POSF and metabolic and environmental degradation of N-alkyl derivatives of perfluorooctanesulfonamide, precursors used in various commercial and consumer application technologies, can lead to the formation of PFOS (Xu et al., 2004, 2006; Schultz et al., 2006; Schröder, 2003). Similarly, the N-alkyl derivatives of perfluorobutanesulfonamides are used in various applications including fabric, carpet, and upholstery protectants,

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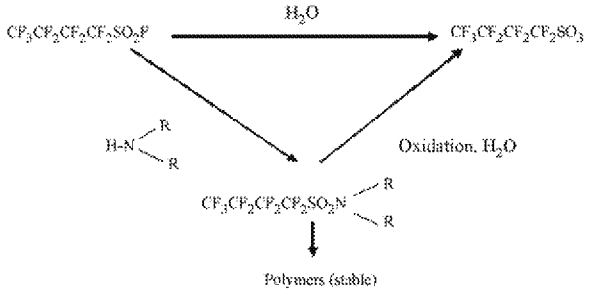

Fig. 1. Outline of the production of PBSF-based materials.

and surfactants. Perfluorobutanesulfonate (PFBS, $C_4F_9SO_3^-$) would be expected to be formed from comparable pathways from PBSF and N-alkyl derivatives of perfluorobutanesulfonamides (Fig. 1). Atmospheric degradation of N-methyl perfluorobutanesulfonamidoethanol has been shown to produce among other degradation products, PFBS (D'eon et al., 2006).

PFOS has a serum elimination half-life of approximately 7 days in rats (Johnson et al., 1979a), a mean of 132 ± 13 days (SD) days in male monkeys and 110 ± 26 days in female monkeys (Noker and Gorman, 2003a), and a geometric mean of 1751 days [95% confidence interval (CI) 1461–2099] days in humans (Olsen et al., 2007). These species-specific differences in serum elimination half-lives have been hypothesized to be governed by a saturable renal resorption process (Andersen et al., 2006). On the other hand, PFBS is likely to have a much faster serum elimination rate in these species due to its shorter perfluorinated chain and/or higher percentage of free PFBS concentration in serum than compared to PFOS (Kerstner-Wood et al., 2003).

To date, only mild effects have been observed in repeat-dose studies with PFBS in rats at relatively high dose levels compared to PFOS. In a 90-day study of potassium PFBS (K^+PFBS) in rats by oral gavage, observed effects included decreased red blood cell count, hemoglobin, and hematocrit at 200 and 600 mg/kg and, in kidneys, minimal to mild papillary/medullary epithelial tubular and ductal hyperplasia in addition to limited interstitial edema and focal necrosis (Lieder et al., 2009). As may be expected due to its structure and chemical properties, PFBS did not cause mutations, chromosomal aberrations, or contact sensitization in standard test systems (Paul Lieder, personal communication).

The differences in toxicological data between PFOS and PFBS suggest that PFBS might have more rapid elimination kinetics. We undertook a series of studies to: (1) determine the extent of urine and fecal elimination of PFBS in rats after administration of single intravenous (i.v.) and oral doses; (2) estimate the pharmacokinetic parameters of PFBS in rats after single intravenous (i.v.) and oral doses; (3) estimate pharmacokinetic parameters of PFBS in cynomolgus monkeys after a single i.v. dose; and (4) estimate the serum elimination half-life of PFBS among workers who manufactured its potassium salt.

2. Materials and methods

2.1. Overview

Studies were completed over a 6-year time frame in the following chronological order: monkeys, humans (pilot and main study), and rats. Analytical methods changed by time period of the study. Analyses were completed in two different bioanalytical laboratories, depending on the study segment. Both laboratories were experienced in the analysis of perfluorinated chemistries; however, no interlaboratory validation studies were conducted between the laboratories. Study samples were analyzed by high performance liquid chromatography mass spectrometry (LC–MS) methods.

The cynomolgus monkey study was completed at Southern Research Institute laboratories using an Applied Biosystems-Sciex model API 3000 mass spectrometer (Applied Biosystems/MDS-Sciex Instrument Corporation, Foster City, CA). This system was operated in the negative ion mode with parent ion (Q1) detection only. The extraction was based on an ion-pairing extraction method using a non-labeled internal standard, perfluoropentanoate (PFPnA). For PFBS, the negative ion monitored was at 299 atomic mass units (amu). For the internal standard PFPnA, the negative ion monitored was 219 amu.

The human pilot- and main-study samples were analyzed at the 3M Medical Department Bioanalytical Laboratory using a Finnigan TSQ 7000 mass spectrometer (ThermoFinnigan, San Jose, CA). This system was operated in the negative ion mode using parent ion (Q1) detection only. The extraction was based on an ion-pairing extraction method and used a non-labeled internal standard, perfluorohexanesulfonate (PFHxS). The negative ions monitored were 299 amu for the PFBS and 399 amu for the internal standard. For both phases of the human studies, the sample extraction method was based on an acidic pH primary extraction followed by a basic pH, reverse extraction method.

The rat pharmacokinetic study samples were analyzed at the 3M Medical Department Bioanalytical Laboratory using an Applied Biosystems-Sciex model API 4000 mass spectrometer. The API 4000 was operated in the negative ion mode using LC–MS/MS tandem mass spectrometry. A new solid-phase extraction (SPE) method was developed using stable-isotope-labeled PFBS [$^{18}O_2$-PFBS, $(CF_3(CF_2)_3S(^{18}O_2)O^-)$, Research Triangle Institute, Research Triangle Park, NC] as an internal standard. The transitions monitored were 299 → 80 amu with PFBS, and 303 → 84 amu with stable-labeled $^{18}O_2$-PFBS internal standard.

For the three species studied, the analytical methods had the following features in common. First, internal standards were added to all extraction tubes (samples, blanks, and controls) prior to extraction. Second, matrix-matched standard curves were prepared by spiking PFBS into the appropriate matrix. The ranges of the standard curves were from 0.5 to 500 ng/mL of PFBS. Third, overall recoveries were greater than 80% and less than 120% of theoretical for all methods employed. Fourth, when a given sample exceeded the high standard, the sample was diluted with matrix-matched material, re-extracted, and then re-analyzed such that the quantitation was accomplished within the range of the standard curve. More specific details of methods employed for analysis of the various samples follow in the appropriate sections for each species studied.

2.2. Pharmacokinetic studies in rats

2.2.1. Materials

All chemicals used in the pharmacokinetic studies in rats were reagent-grade and were purchased from Sigma–Aldrich (St. Louis, MO) or VWR (West Chester, PA). K^+PFBS (98.2% pure) was supplied by the 3M Company (St. Paul, MN).

2.2.2. Laboratory animals and animal care

Male and female Sprague–Dawley (SD) rats (8–10 weeks old, 200 g–250 g) were purchased from Charles River Laboratory (Portage, MI). All rats were housed in standard cages. Teklad Mouse/Rat Chow and tap water were provided to all rats *ad libitum* throughout the study except when fasting was required. Environmental controls for the animal room were set to maintain a temperature of 22.2 ± 1.7 °C, humidity of 30–70%, a minimum of 10 exchanges of room air per hour and a 12 h light/dark cycle. Studies were performed in 3M facilities accredited by the Association for Assessment and for the Accreditation of Laboratory Animal Care International. All procedures involving rats were reviewed and approved by the Institutional Animal Care and Use Committee. Animal care and procedures followed the U.S. Department of Health and Human Services Guide for the Care and the Use of Laboratory Animals (Institute of Laboratory Animal Resources, 1996).

2.2.3. Experimental designs

In the three rat studies described below, serum samples were obtained after blood clotting and centrifugation (2000 × *g*, 15 min). All samples taken, including serum, urine, feces, and liver, were frozen with liquid nitrogen and stored at −80 °C pending analysis for PFBS by LC–MS/MS. Dosing solution concentration and homogenicity analyses were not performed.

2.2.3.1. Urinary and fecal elimination study in rats. Male and female Sprague–Dawley rats (n = 3/sex) were given a single dose of 30 mg K^+PFBS/kg body weight by either intravenous injection (via tail vein) or oral gavage. The K^+PFBS solution was prepared in vehicle (saline or deionized water for i.v. and oral routes, respectively). Immediately after dosing, rats were placed in metabolism cages, and urine and fecal samples were collected every 24 hours (h) for 96 h post-dose. At the end of 96 h, rats were euthanized via CO_2 asphyxiation, blood (collected via abdominal aorta) and liver samples were harvested.

2.2.3.2. Intravenous dose pharmacokinetic study in rats. Male and female Sprague–Dawley jugular-cannulated rats (n = 3/sex) were given a single i.v. injection (via tail vein) of 30 mg K^+PFBS/kg body weight. The PFBS solution was prepared in saline. Interim blood samples (approximately ~0.5 mL) were collected from cannula at 0.25, 0.5, 1, 2, 4, 8, 18, and 24 h post-dose.

2.2.3.3. Oral dose uptake and elimination study in rats. Male and female Sprague–Dawley jugular-cannulated rats ($n = 3$/sex, fasted overnight) were given a single oral dose of 30 mg K^+PFBS/kg body weight. The dosing solution was prepared in deionized water. Interim blood samples (approximately ~0.5 mL) were collected from cannula at 0.25, 0.5, 1, 2, 4, 8, 18, and 24 h post-dose.

2.2.4. LC–MS/MS analysis of samples

2.2.4.1. Sample preparation: serum, urine, liver, and feces. Serum and urine samples were used as is and no further preparation was necessary. Livers from naïve rats (used as blank matrix), PFBS-treated, and vehicle-treated rats were stored at −80 °C until ready for analysis. Samples were allowed to thaw, and approximately 0.2 g of liver was weighed and homogenized with deionized water in a clean polypropylene tube. The ratio between liver and water was 1:4 (w/w). After the primary homogenization step, the whole homogenate was further sonicated for 30 min.

Fecal samples from naïve rats (used as blank matrix), PFBS-treated, and vehicle-treated rats were stored at −80 °C until analysis. Samples were allowed to thaw and then the entire fecal sample for each rat was weighed and homogenized with deionized water in a clean polypropylene tube. The ratio between feces and water was 1:3 (w/w). After the primary homogenization step, the whole homogenate was centrifuged at 2500 × *g* for 20 min and the corresponding supernatant was referred to as fecal extract.

To prepare a LC–MS/MS PFBS standard curve, a PFBS solution prepared in methanol was aliquoted volumetrically to clean polypropylene tubes. 100 μL of the appropriate blank matrix (serum, urine, liver or fecal extract) was added to each tube. The final concentrations of PFBS were 0, 2.5, 5, 12.5, 25, 50, 75, and 100 ng/mL.

2.2.4.2. Solid phase extraction (SPE): serum, urine, fecal extract, and liver. For each matrix (serum, urine, fecal extract or liver homogenate), 100 μL standards and test samples were aliquoted into clean polypropylene tubes, followed by the addition of $^{18}O_2$-labeled PFBS internal standard. One milliliter of 1.0N formic acid was added to all tubes, followed by 100 μL saturated ammonium sulfate (serum only). Samples were vortexed between each addition. All extractions utilized Waters Oasis® hydrophilic–lipophilic balance (HLB) 3 mL columns (Waters Corporation, Milford, MA) with column conditioning, column loading, column wash, and column elution performed as described by Ehresman et al. (2007).

2.2.4.3. LC–MS/MS conditions. The instrument used for analysis was an API 4000 mass spectrometer (Applied Biosystems/MDS-Sciex Instrument Corporation, Foster City, CA) configured with Turbo Ion Spray (pneumatically assisted electrospray ionization source) in negative ion mode. A Mac-Mod ACE® C-18, 5 μm, 100 × 2.1 mm i.d. HPLC column with an isocratic flow rate of 0.35 mL/min was used for PFBS analysis. The mobile phase was 51% acetonitrile and 49% 2 mM ammonium acetate. All source parameters were optimized under these conditions according to manufacturer's guidelines.

2.2.5. Pharmacokinetic data analysis

Selected pharmacokinetic parameters were calculated from the serum PFBS concentration versus time data using WinNonlin (Professional Version 4.1; Pharsight Corp.; Mountain View, CA). Data obtained after i.v. or p.o. dosing were fit to a two compartment model with first order elimination. A weighting factor of $1/y^2$ was applied to each data set.

Statistically significant ($p < 0.05$) differences in the sex-specific arithmetic means for each pharmacokinetic parameter were determined by the student's *t*-test.

2.3. Intravenous dose pharmacokinetic study in monkeys

2.3.1. Materials

K^+PFBS was supplied by 3M Company (St. Paul, MN). The test article was stored at room temperature until used. The vehicle used for the preparation of the dose formulation was sterile saline, USP (Phoenix Pharmaceutical Company, St. Joseph, MO; Lot 8101069) and was stored at room temperature. The formulation (5 mg/mL in saline) was stored refrigerated and used for dosing within 3 days after preparation; it was considered to be stable during this period. Dosing solution concentration and homogeneity analyses were not performed.

2.3.2. Laboratory animals and animal care

The three male and three female cynomolgus monkeys designated for use in this study were selected from an in-house colony of monkeys that were housed at Southern Research Institute (Southern Research, Birmingham, AL) prior to use on this study. These monkeys were purchased from Charles Rivers BRF, Inc. (Houston, TX). Animal care and procedures followed the US Department of Health and Human Services Guide for the Care and the Use of Laboratory Animals (Institute of Laboratory Animal Resources, 1996), the U.S. Department of Agriculture through the Animal Welfare Act (Public Law 99-198) and to the applicable Standard Operating Procedures (SOPs) of Southern Research Institute. The Southern Research Institute Animal Care and Use Committee reviewed and approved the study protocol.

Certified, commercial, dry monkey chow #5048 (PMI Feeds, Inc., St. Louis, MO) was fed to the monkeys 2–3 times each day. The diet was supplemented with fresh fruit/treats several times each week. Tap water (Birmingham, Alabama public water supply) was available to the monkeys *ad libitum*. The monkeys were housed in a room that was maintained at a temperature of 20.0–21.3 °C and a relative humidity of 22.2–65.7%. An automatic timer set to provide 12 h of light and 12 h of dark per day controlled room lights.

2.3.3. Experimental design

On Day 0, each of the three male and three female cynomolgus monkeys received a single i.v. dose of K^+PFBS at 10 mg/kg into a superficial arm or leg vein. Doses were administered at a volume of 2 mL/kg based on the Day 0 body weights. All monkeys were observed twice daily for clinical signs. Each monkey was weighed on Days 0, 4, 7, and 14. Urine was collected in standard metabolism cages for 24-h intervals on the following days: prior to dose administration (Day −1; baseline), on Day 1 (0–24 h post-dose), and on Days 7 (144–168 h post-dose) and 14 (312–336 h post-dose). The volume of each urine sample was measured. Urine samples were stored frozen (approximately −20 °C). Fecal samples were also collected but were not analyzed. Blood samples (2 mL) were collected at approximately 0 min (predose); 2, 4, 8, 24, and 48 h; and on Days 4, 7, 11, 14, and 31 post-dose. Samples were collected into tubes without anticoagulant and were allowed to clot at room temperature. The blood samples were then centrifuged, and the serum separated and stored at −20 °C until analyzed.

2.3.4. Sample preparation

Analyses of monkey serum and urine samples were performed at Southern Research Institute. PFBS standards were prepared with the low of 0.25 ng/mL and the high of 500 ng/mL. Monkey serum or urine samples (0.5 mL) were fortified with internal standard PFPnA, followed by the addition of ion-pairing reagent (tetrabutylammonium hydrogen sulfate) and carbonate/bicarbonate buffer, and extracted with ethyl acetate. The ethyl acetate layer was removed by evaporation, and the resulting residue was reconstituted in mobile phase (70% 5 mM ammonium acetate; 30% methanol with 1.5% formic acid). After filtration through a 0.2 μm syringe, the samples were transferred to auto sampler vials and analyzed by LC–MS.

2.3.5. LC–MS conditions

The mass spectrometry (PE Sciex API 3000) of PFBS and PFPnA was accomplished in the negative-ion mode. The ion spray source voltage was set at −2000 V which was low enough to have reduced the formation of other potentially interfering ions extracted from the serum. In addition, the collision energy was set high to reduce potential interferences from other matrix compounds. In order to maximize sensitivity, this method was based on mixed reactions monitoring of the negative parent ion for both PFBS and PFPnA.

2.3.6. Data analysis

Pharmacokinetic parameters were estimated from serum PFBS monkey concentrations using WinNonlin (Version 1.1; Scientific Consulting, Inc.; Apex, NC). The data were fit to a three-compartment model. Mean values and standard deviations for each parameter were calculated. The urinary excretion of PFBS at each collection interval was calculated and expressed as a percent of the administered dose. Statistically significant ($p < 0.05$) differences in the sex-specific arithmetic means for each pharmacokinetic parameter were determined by the student's *t*-test.

2.4. Half-life of serum PFBS elimination studies in humans

2.4.1. Pilot study design

Nine blood samples were collected over a 152-day time period (December 20, 2002 to May 21, 2003) from one white male 3M employee engaged in the production of K^+PFBS. Because K^+PFBS was only manufactured on an approximate semi-annual basis, the potential for occupational exposure was avoided throughout this pilot study by removing this individual from specifically working in the K^+PFBS manufacturing area during the entire study time period. Upon collection of 8 mL of blood on each of the nine collection dates, the sample was allowed to clot in order to obtain split serum samples. Split serum samples were kept at −20 °C until thawed for laboratory analysis. No urine samples were collected during the course of the pilot study.

The pilot study was approved by the 3M Institutional Review Board (IRB). Prior to the initiation of the study, the subject read and signed an informed consent form. The subject received an honorarium of $25 for each blood sample.

2.4.2. Main study design

Upon completion of the pilot study and informed of the pilot study results, six additional 3M employees (five white male, one white female) engaged in K^+PFBS production volunteered to participate in a six-month follow-up study of the serum elimination of PFBS. One employee was the same individual who participated in the pilot study. As stated above, the approximate semi-annual batch production of K^+PFBS resulted in the six participants not having any further occupational exposure until after end-of-study. A total of 10 blood collections occurred for each participant between June 2004 and December 2004. Upon collection of the approximate 8 mL of blood, each sample was allowed to clot in order to obtain two serum samples (split samples) per collection. Spot (non-24 h) urine samples were collected near

the beginning, middle and end-of-study. No fecal samples were obtained. Serum and urine samples were kept at −20 °C until laboratory analysis.

The main study was approved by the 3M IRB. Prior to participating, the six employee subjects read and signed informed consent forms. Participants received an honorarium of $25 for each blood sample and $10 for each urine sample provided.

2.4.3. Sample preparation

2.4.3.1. Pilot study. A series of PFBS standard curves were prepared for LC–MS analyses in the pilot study. The final concentrations of the PFBS standards were 0, 2.5, 5, 10, 25, 50, 75 and 100 ng/mL. To each 0.5 mL of unknown test sample or standard, 1.0 mL of ion-pairing reagent (tetrabutylammonium hydrogen sulfate, pH 10.0) was added followed by the addition of 1.0 mL of carbonate/bicarbonate and 1.0 mL of deionized water. This solution was vortexed followed by a subsequent addition of 5 mL ethyl acetate. After mixing on a mechanical shaker for 1 h, all solutions were centrifuged at 2500 × *g* for 5 min. Four milliliters of the ethyl acetate layer (top layer) was volumetrically transferred to a clean polypropylene tube containing 10 μL of 5 ng/μL (50 ng) of perfluorohexanesulfonate (PFHxS) as a quantitation reference standard. The ethyl acetate was then removed under a gentle stream of nitrogen gas at 45 °C using an N-Evap drier. The remaining residue was re-suspended in 250 μL of 25% acetonitrile and 75% 10 mM ammonium acetate buffer. This mixture was then filtered through a 0.2 μm syringe filter and transferred to a HPLC vial from which 20 μL was injected to the LC–MS system. The HPLC column used a Mac-Mool ACE, C-18 based deactivated column, 5 μm, 2.1 mm i.d. by 100 mm. The flow was isocratic at 0.250 mL/min with 30% acetonitrile and 70% 10 mM ammonium acetate mobile phase. The Finnigan TSQ system was operated in the electro spray ionization mode using negative ionization detection with a constant source potential of 3.0 kV applied.

2.4.3.2. Main study. PFBS standards were prepared ranging from 10–400 ng/mL. For each unknown control or blank serum sample, 250 μL of sample was pipetted into a polypropylene test tube already containing the appropriate amount of internal standard (PFHxS). To each tube were added 300 μL of 1.0N formic acid and 300 μL of saturated ammonium sulfate and all tubes were mixed well by vortexing prior to the addition of 5.0 mL of acetonitrile. The samples were then extracted by shaking the tubes on a mechanical shaker for 30 min followed by centrifugation for 5 min at 2500 × *g*. After centrifugation, the organic phase was transferred to a new, clean polypropylene tube which was then evaporated using a LabConCo Rapid Vac Vacuum System (LabConCo Corporation, Kansas City, MO). The remaining residue was reconstituted with 300 μL of deionized water and the pH was adjusted to 12 with the addition of 300 μL of 1.0N potassium hydroxide. All tubes were vortexed and 7.0 mL of methyl-1-butyl-ether was added. Extraction into the ether phase was completed by shaking all tubes on a mechanical shaker for 20 min followed by centrifugation for 5 min at 2500 × *g*. The ether layer (top layer) was transferred in total to a new, clean, polypropylene tube and evaporated under a gentle stream of nitrogen gas. The residual material was then dissolved in 200 μL of 50% acetonitrile and 50% 10 mM ammonium acetate and transferred to a polypropylene HPLC micro-vial assembly from which 5 μL sample was injected into the LC–MS system for analysis. The LC–MS used for the PFBS analysis during the main study was the same Finnigan system used for the pilot study. The same HPLC column and conditions were used as previously described.

2.4.4. Data analysis

Selected pharmacokinetic parameters were calculated from the mean serum PFBS concentration versus time data using WinNonlin (Professional Version 4.1; Pharsight Corp.; Mountain View, CA). Data were fit to a first-order (WinNonlin Model 1) or in the case of the pilot study, to a second order model. A weighting factor of $1/y$ was applied to each data set.

3. Results

3.1. 3Pharmacokinetic studies in rats

3.1.1. Urinary and fecal elimination study in rats

The limit of quantitations (LOQ) for urine, feces, liver, and serum concentrations in rats were 0.010 μg/mL, 0.040 μg/g, 0.050 μg/g, and 0.010 μg/mL, respectively. Twenty-four hours after an intravenous administered dose of 30 mg/kg K^+PFBS to three male and three female rats, 66.3% [Standard Error (SE) ± 8.7] and 74.4% (±6.0) of the dose was determined to be in the urine, respectively (Table 1). Only 0.36% (±0.09) and 0.13% (±0.03) of the dose, respectively, was found in the feces after 24 h. At 96 h post-dose, the percent of dose in feces and liver could not be determined because measurements were below LOQ except for one male rat where the percent of dose in feces was 0.003%. Except for one male rat whose serum PFBS concentration was 0.028 μg/mL at 96 h post-dose, the other male and female rats had serum PFBS concentrations at the LOQ (0.010 μg/mL) at 96 h post-dose. All liver PFBS concentrations were below the LOQ (0.050 μg/g) at 96 h post-dose.

Table 1
Mean ± SE values for serum and liver concentrations of PFBS and percent of given single oral or i.v. dose (30 mg K^+PFBS/kg body weight) in urine, feces, and liver up to 96 h after dosing in Sprague–Dawley rats.

		Oral	i.v.
% of dose in urine at 24 h	Male	68.6 ± 12.9	66.3 ± 8.7
	Female	74.1 ± 8.2	74.4 ± 6.0
% of dose in urine at 96 h	Male	0.27 ± 0.21	0.10 ± 0.05
	Female	0.30 ± 0.16	0.08 ± 0.03
% of dose in feces at 24 h	Male	0.50 ± 0.17	0.36 ± 0.09
	Female	0.14 ± 0.07[a]	0.13 ± 0.03
% of dose in feces at 96 h	Male	0.04 ± 0.04[b]	0.003[c]
	Female	0.003[d]	N/A[e]
% of dose in liver at 96 h	Male	0.03 ± 0.003	N/A[f]
	Female	0.05[g]	N/A[f]

[a] Excludes data for an individual female for which there was evidence that spillage of urine into the fecal collection cup had occurred.
[b] Excludes data for one male that had feces measured at <LOQ (0.040 μg/g).
[c] Excludes data for two males that had feces measured at <LOQ (0.040 μg/g).
[d] Excludes data for two females that had feces measured at <LOQ (0.040 μg/g).
[e] All fecal samples for females in i.v. study were measured at <LOQ (0.40 μg/g).
[f] All liver samples in i.v. study (male and female) were measured <LOQ (0.050 μg/g).
[g] Excludes data for two females that had liver measured at <LOQ (0.050 μg/g).

After an oral administered dose of 30 mg/kg K^+PFBS to three male and three female rats, 68.6% (±12.9) and 74.1% (±8.2) of the dose were determined to be in the urine after 24 h, respectively (Table 1). In these rats, 0.5% (±0.17) and 0.14% (±0.07) of the administered dose was found in the feces after 24 h, respectively. Mean serum and liver PFBS concentrations at 96 h post-dose for male rats were 0.028 (±0.014) μg/mL and 0.134 (±0.013) μg/g, respectively. Among females, one rat had a measured serum concentration of 0.012 μg/mL PFBS at 96 h post-dose while the other two were reported below LOQ (0.010 μg/mL). One female rat had a liver concentration of 0.343 μg/g at 96 h post-dose while the other two animals were a reported below LOQ (0.050 μg/g).

Although it was not feasible to measure 24 h post-dose serum PFBS concentrations in the above two urinary and fecal elimination studies, estimates can be derived from the intravenous and oral dose uptake elimination studies that follow (see Table 2) as the same administered dose and type of rat were used.

3.1.2. Intravenous dose pharmacokinetic study in rats

Presented in Table 2 are the pharmacokinetic parameters calculated from serum concentrations of PFBS in three male and two female jugular-cannulated rats that received a single i.v. dose of 30 mg K^+PFBS/kg body weight. Although three female rats were dosed, one female rat was excluded from the analysis because a blood sample at 18 h could not be obtained due to a clotted cannula. A two-compartmental model best fit the data (Fig. 2). In male and female rats, mean C_{max} data were 142 ± 22 and 120 ± 6 μg/mL, respectively. Mean serum PFBS concentration at 24 h for male rats was 0.57 ± 0.51 μg/mL, a value which was higher than the mean for female rats at 0.09 ± 0.05 μg/mL but not statistically significant. The mean initial ($T_{0.5}\alpha$) serum elimination half-life was nonsignificantly greater in males (0.99 h) than females (0.36 h) while the β-phase terminal ($T_{0.5}\beta$) serum elimination half-lives of 4.51 and 3.96 h, in male and female rats, respectively, were not significantly different. Male rats had a significantly lower mean clearance (CL) (119 mL/h) than females (469 mL/h) and a significantly larger mean area under the curve (AUC) (294 μg·h/mL vs. 65 μg·h/mL). The vol-

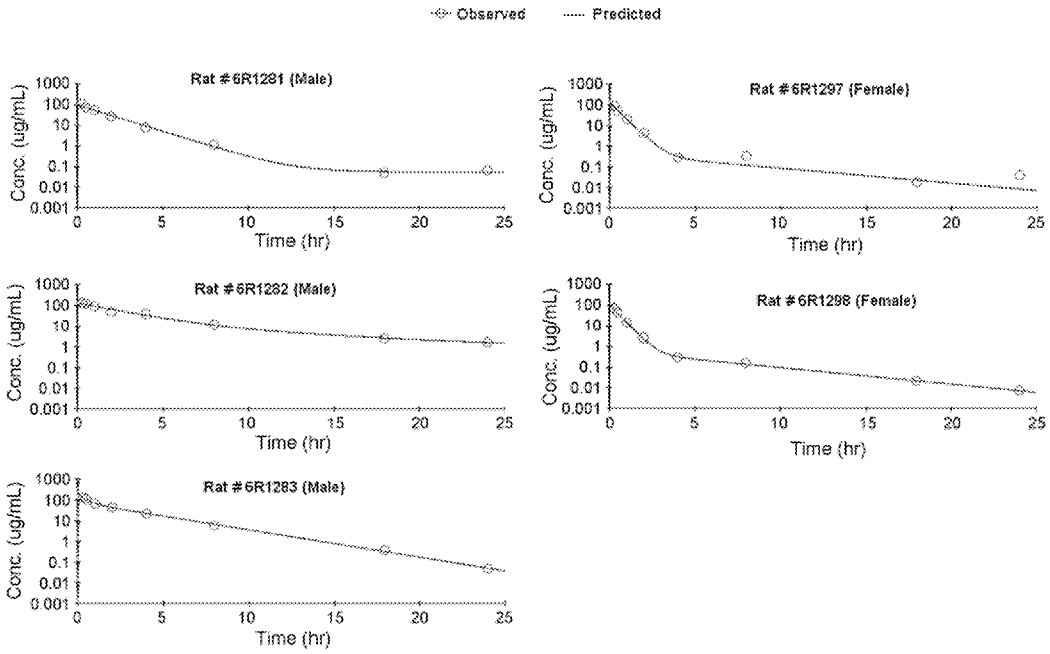

Fig. 2. Serum perfluorobutanesulfonate (PFBS) concentrations (μg/mL) in male (left column) and female (right column) Sprague–Dawley rats after a single i.v. dose of 30 mg K^+PPFBS/kg body weight.

ume of distribution (V_{dss}) was similar between males and females (330 mL/kg vs. 351 mL/kg) and suggested predominant extracellular distribution.

3.1.3. Oral dose uptake and elimination study in rats

Also presented in Table 2 are the pharmacokinetic parameters for three male and three female jugular-cannulated rats that received a single oral dose of 30 mg K^+PFBS/kg body weight. As with the i.v. data, a two-compartmental model fit the data (Fig. 3). In male and female rats, mean T_{max} values were 0.42 and 0.33 h, respectively, and C_{max} values were 86 ± 5 and 102 ± 15 μg/mL, respectively. The mean serum PFBS concentrations at 24 h were statistically significantly higher in males (0.38 ± 0.07 μg/mL) than females (0.02 ± 0.01 μg/mL). The terminal mean half-life of serum elimination was significantly shorter in males (4.68 ± 0.43 h) than females (7.42 ± 0.79 h), yet the mean AUC was significantly greater in males (163 ± 10 μg·h/mL) than females (85 ± 12 μg·h/mL). The lower AUC in females may have been due to the initial rapid elimination.

Table 2
Mean ± SE values for pharmacokinetic parameters in Sprague–Dawley rats given either a single oral or a single i.v. dose of 30 mg K^+PFBS/kg body weight.

Parameter	Sex	Oral	i.v.
T_{max} (h)	Male	0.42 ± 0.08	N/A[a]
	Female	0.33 ± 0.08	N/A
C_{max} (μg/mL)	Male	86 ± 5	142 ± 22
	Female	102 ± 15	120 ± 6
Serum [PFBS] @ 24 h (μg/mL)	Males	0.38 ± 0.07	0.57 ± 0.51
	Female	0.02 ± 0.01**	0.09 ± 0.05
λ_α (h^{-1})	Male	0.892 ± 0.079	1.143 ± 0.560
	Female	1.308 ± 0.020**	1.956 ± 0.083
λ_β (h^{-1})	Male	0.151 ± 0.015	0.228 ± 0.075
	Female	0.095 ± 0.009*	0.175 ± 0.009
$T_{0.5}\alpha$ (h)	Male	0.79 ± 0.07	0.99 ± 0.43
	Female	0.53 ± 0.01*	0.36 ± 0.02
$T_{0.5}\beta$ (h)	Male	4.68 ± 0.43	4.51 ± 2.22
	Female	7.42 ± 0.79*	3.96 ± 0.21
CL (mL/h)	Male	N/A	119 ± 34
	Female	N/A	469 ± 40**
AUC (μg·h/mL)	Male	163 ± 10	294 ± 77
	Female	85 ± 12**	65 ± 5
V_{dss} (mL/kg)	Male	676 ± 55	330 ± 32
	Female	391 ± 105	351 ± 34

[a] Not applicable.
* Significantly different from the male group value ($p \leq 0.05$).
** Significantly different from the male group value ($p \leq 0.01$).

3.2. Intravenous dose pharmacokinetic study in monkeys

Presented in Table 3 are pharmacokinetic parameters in cynomolgus monkeys upon the administration of a single i.v. dose of 10 mg/kg K^+PFBS. There were no statistically significant differences by sex for any of the parameters presented in Table 3. Mean serum concentrations at 24 h for males and females were 5.68 ± 2.42 and 9.56 ± 4.91 μg/mL, respectively. PFBS was not quantifiable in serum at Day 31. The LOQ for monkey serum and urine for

Table 3
Mean ± SE pharmacokinetic parameters calculated from serum concentration of monkeys receiving a single i.v. 10 mg/kg dose of K^+PFBS.

Parameter	Males	Females
Serum [PFBS] (μg/mL) at 2 h	32.20 ± 6.31	55.03 ± 6.55
Serum [PFBS] (μg/mL) at 24 h	5.68 ± 2.42	9.56 ± 4.91
Serum [PFBS] (μg/mL) at 168 h	0.029 ± 0.021	0.30 ± 0.29
$T_{0.5}\alpha$ (h)	0.80 ± 0.29	1.28 ± 0.16
$T_{0.5}\beta$ (h)	13.20 ± 2.88	11.28 ± 2.51
$T_{0.5}\gamma$ (h)	95.20 ± 27.09	83.20 ± 41.90
AUC (μg·h/mL)	24.3 ± 8.6	35.4 ± 13.3
CL (mL/h)	511 ± 141	368 ± 120
V_{dss} (mL/kg)	254 ± 31	255 ± 17
Urine [PFBS] (μg/mL) at 24 h	100.48 ± 24.53	24.41 ± 11.33
Urine [PFBS] (μg/mL) at 168 h	0.15 ± 0.03	0.22 ± 0.10
% of dose in urine from 0 to 24 h	48.9 ± 7.9	44.4 ± 24.9
% of dose in urine from 144 to 168 h	0.15 ± 0.07	0.18 ± 0.12

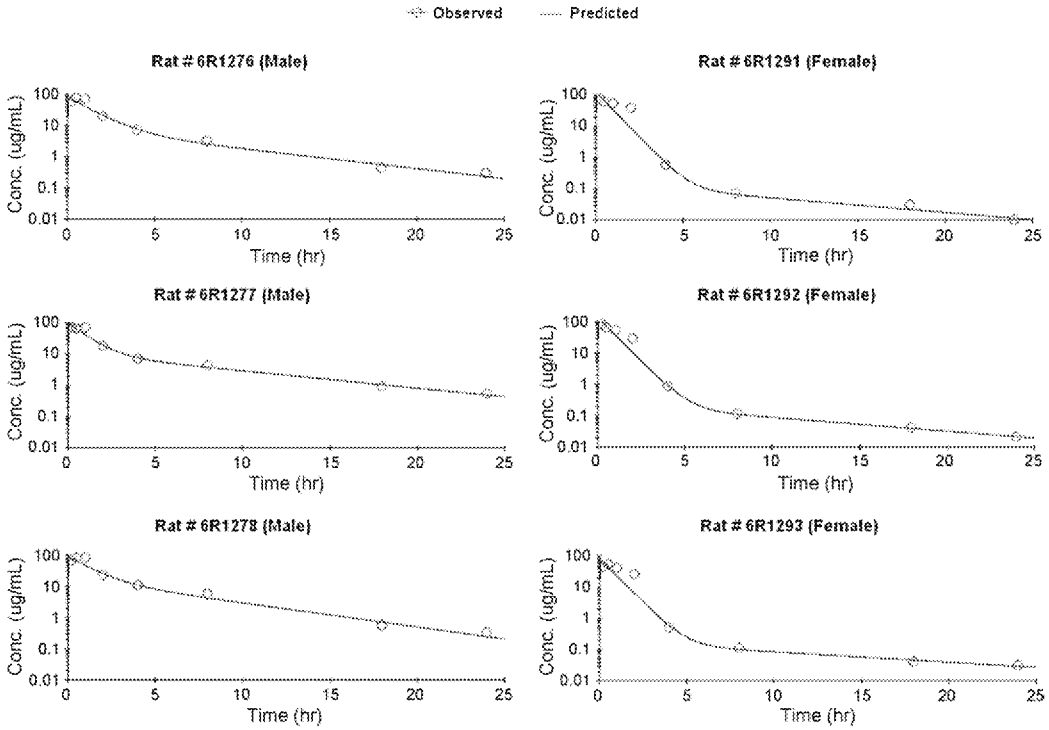

Fig. 3. Serum perfluorobutanesulfonate (PFBS) concentrations (μg/mL) in male (left column) and female (right column) Sprague–Dawley rats after a single oral dose of 30 mg K^+PFBS/kg body weight.

PFBS were 0.5 ng/mL (0.0005 μg/mL) and 1.0 ng/mL (0.001 μg/mL), respectively.

For five monkeys, 33.8–86.8% of the dose was recovered in the urine within 24 h after administration of K^+PFBS. For the remaining monkey, less than 1% of the dose was recovered in urine during this same time interval. The serum PFBS concentrations from that particular monkey indicated the animal had been properly dosed. Loss or spillage of urine excreted most likely occurred during the collection interval although this cannot be positively confirmed. With time after dosing, the amount of PFBS detected in the urine decreased among all monkeys (data not shown). By Day 14 (312–336 h post-dose), individual monkeys excreted less than 0.01% of the dose within a 24 h interval.

Concentration versus time data were best fit to a three compartmental model (Fig. 4). Mean serum elimination half-lives for PFBS in the male monkeys were 0.8, 13.2, and 95.2 h for the α, β, and γ phases, respectively, with the corresponding values in the female monkeys of 1.28, 11.28, and 83.2 h (Table 3). Although not statistically significantly different between sexes, the mean value for CL was lower in females and mean AUC was higher. The mean V_{dss} was nearly identical for both sexes and suggested predominantly extracellular distribution.

3.3. Serum elimination studies in humans

3.3.1. Pilot study

The initial (Day 0) serum sample PFBS concentration was 945 ng/mL (0.945 μg/mL). The end-of-study (Day 152) serum sample PFBS concentration was 7.5 ng/mL (0.0075 μg/mL). The LOQ for serum in the pilot study was 5 ng/mL (0.005 μg/mL). A two-compartment model best fit the data (Fig. 5). The serum elimination half-lives were 11.6 and 39.4 days, for the α and β phases, respectively.

3.3.2. Main study

The mean serum PFBS concentration at study onset was 397 ng/mL (0.397 μg/mL) ± 115 (SE) with the range between 92 and 921 ng/mL. The median PFBS concentration was 363 ng/mL. Determination of the serum elimination half-lives for the six subjects included all serum PFBS concentrations measured above the LOQ of 5 ng/mL (0.005 μg/mL). The data best fit a one-compartment model (Fig. 6). R squares ranged from 0.982 to 0.998. Subject #1 was the same individual who was the sole participant in the pilot study. Subject #6 was the female employee. The mean serum elimination half-life for PFBS was 27.7 days (95% confi-

Table 4
Paired [PFBS] serum and urine concentrations (ng/mL) by human subject and estimated serum elimination half-life ($T_{0.5}$) (days).

Subject	Study onset		First pair			Second pair			Last pair			$T_{0.5}$ (days)
	Day	Serum	Day	Serum	Urine	Day	Serum	Urine	Day	Serum	Urine	
Subject 1	0	405	5	354	173	60	71	14	180	LOQ*	LOQ**	24.5
Subject 2	0	92	2	88	12	49	19	LOQ	175	LOQ	LOQ	21.2
Subject 3	0	239	8	166	110	57	16	6	175	LOQ	LOQ	13.1
Subject 4	0	347	5	313	5	60	108	9	180	7	LOQ	32.5
Subject 5	0	921	5	725	102	62	190	12	180	25	LOQ	29.2
Subject 6	0	378	5	361	39	62	167	LOQ	180	20	LOQ	45.7

* Limit of quantitation in serum (5 ng/mL).
** Limit of quantitation in urine (5 ng/mL).

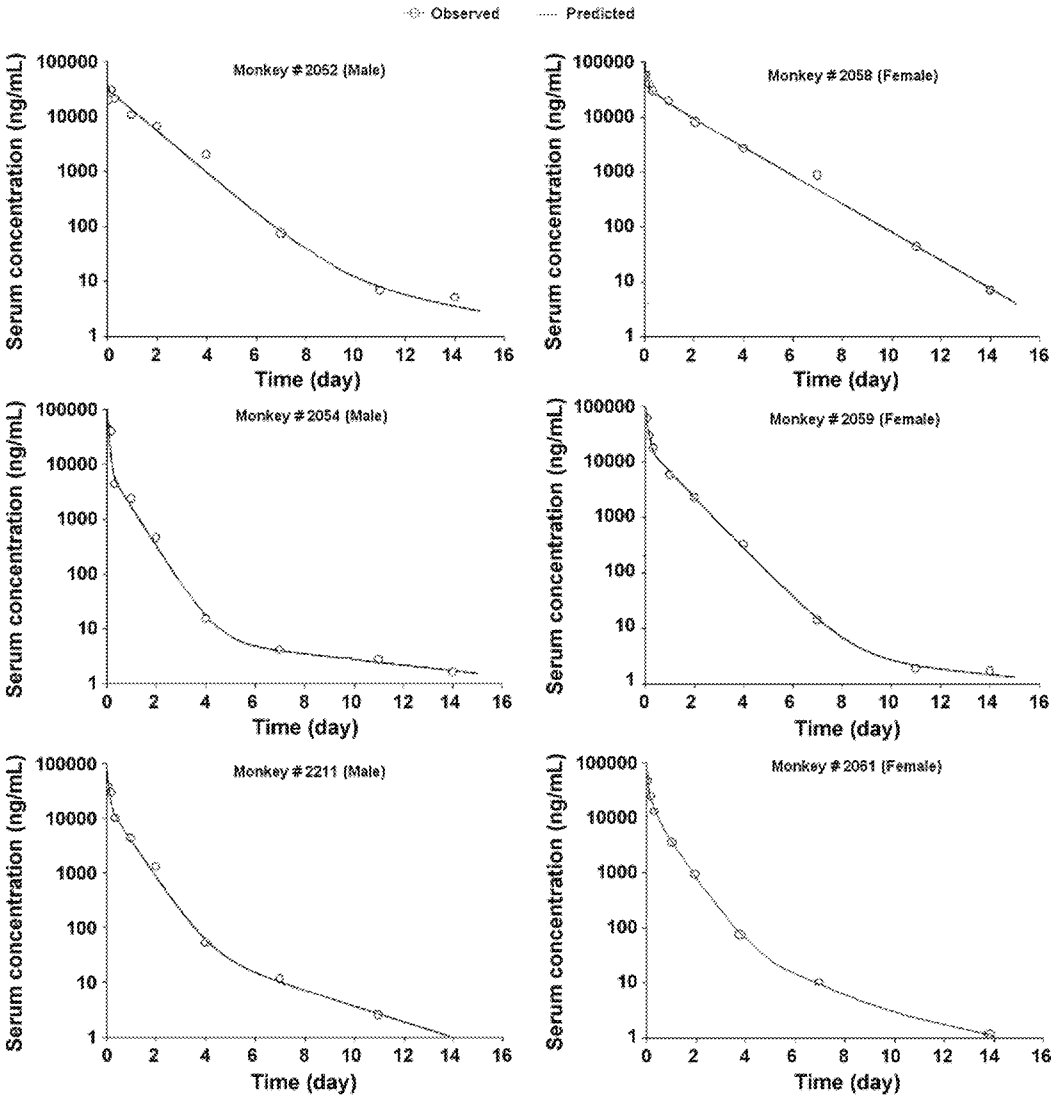

Fig. 4. Serum perfluorobutanesulfonate (PFBS) concentrations (ng/mL) in male (left column) and female (right column) cynomolgus monkeys after a single i.v. dose of 10 mg K^+PPFBS/kg body weight.

dence interval 16.1–39.3). The geometric mean serum elimination half-life was 25.8 days (95% CI 16.6–40.2). Among the five male subjects, the mean serum half-life of elimination was 24.1 days (95% CI 13.1–32.5) and the geometric mean was 23.0 days (95% CI 14.8–35.8). The female employee had the longest serum elimination half-life of 45.7 days. Urine was a route of elimination of PFBS as concentrations early in the study ranged from 5 to 173 ng/mL and declined during the study such that all measurements were less than the LOQ (5 ng/mL) by end-of-study (Table 4). Spearman Rho coefficients for the first and second paired serum and urine samples were 0.26 ($p = 0.62$) and 0.23 ($p = 0.66$).

4. Discussion

The results from this series of studies have established pharmacokinetic parameters of PFBS for the rat and monkey including C_{max}, $T_{0.5}$, CL, AUC, and V_{dss}, and have estimated $T_{0.5}$ of PFBS in the human. Based on the values estimated for the V_{dss} in the rat and monkey, PFBS appears to be distributed predominantly in extracellular space. Based on i.v. doses, the V_{dss} values for male and female rats, given 30 mg K^+PFBS/kg, and male and female monkeys given 10 mg K^+PFBS/kg, were 330 and 351, and 254 and 255 mL/kg, respectively.

Urine appeared to be a major route of elimination in the rat and monkey for PFBS. Rats and monkeys of both sexes excreted approximately one-half to three-quarters of the single administered oral (rat) and i.v. (rat and monkey) doses of PFBS over the initial 24-h period. This declined to less than 0.5% by 96 h. Among the six human subjects in the main study, serum concentrations

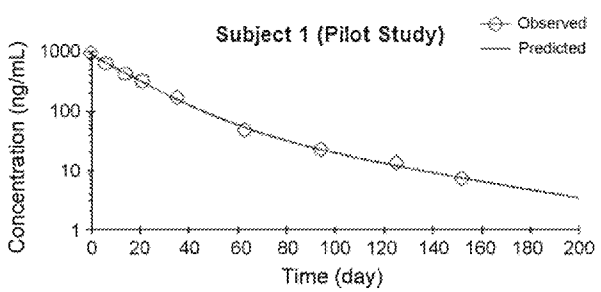

Fig. 5. Pilot human study: serum PFBS concentrations (ng/mL) by time in one male worker who manufactured K^+PPFBS.

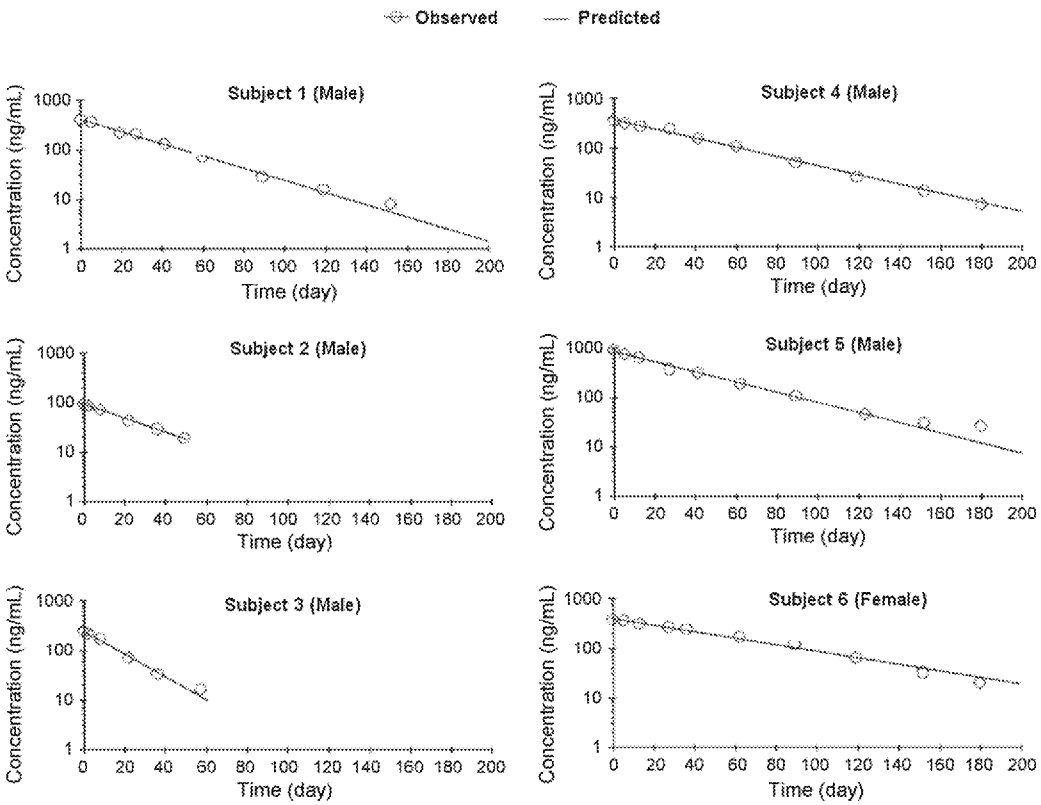

Fig. 6. Main human study: serum PFBS concentrations (ng/mL) by time in six workers (five male, one female) who manufactured K^+PPFBS.

were modestly correlated with paired urine concentrations and both concentrations declined over time such that at end-of-study urine was measured at the LOQ for the six subjects.

The rat serum PFBS elimination rates reported herein are markedly faster than the respective serum elimination rate that has been observed for the eight-carbon homolog PFOS. Johnson et al. (1979a) studied the elimination of ^{14}C from ^{14}C-labeled K^+PFOS in the urine and feces of six male rats following a single i.v. dose of 10.8 mg/kg. They observed that 42.8% of the ^{14}C was eliminated in urine and feces over an 89-day period following the i.v. dose. The serum PFBS elimination $T_{0.5}$ in rats of approximately 4 h reported herein is about 600-fold less than the whole-body elimination $T_{0.5}$ for PFOS of about 100 days that can be approximated based on the Johnson et al. (1979a) study. An estimated serum elimination half-life of 7.5 days following a single oral dose of ^{14}C-labeled potassium PFOS was also reported by Johnson et al. (1979b); however, in that study, elimination of ^{14}C was followed only in the serum, and not in urine and feces. Considering both the i.v. and oral studies of Johnson et al., it is likely that the shorter serum elimination $T_{0.5}$ for PFOS based on the oral study may have been an underestimate of whole body elimination possibly due to initial more rapid phase elimination of PFOS and/or redistribution within the body. Indeed, Johnson et al. (1984) provided evidence for enterohepatic circulation of PFOS, and Johnson et al. (1979a) and Seacat et al. (2003a,b) have noted significant uptake of PFOS in the liver of rats following single i.v. dosing (Johnson et al., 1979a) or repeated dietary dosing (Seacat et al., 2003a,b). No data are available on the serum elimination half-life of PFHxS in rats.

The $T_{0.5}$ for PFBS in male and female monkeys (approximately 4 days) was 30-fold less than the mean $T_{0.5}$ observed for PFOS (males 132 days and females 110 days) (Noker and Gorman, 2003a). A similar $T_{0.5}$ ratio was seen between PFBS and PFHxS (male monkeys 141 days and female monkeys 87 days) (Noker and Gorman, 2003b).

Humans consistently have had longer serum elimination half-lives compared to other studied species for PFBS, PFHxS or PFOS. The geometric mean serum elimination half-life of PFBS was 25.8 days (95% CI 16.6–40.2) compared to a PFOS geometric mean of 1751 days (95% CI 1461–2099) and a PFHxS geometric mean of 2662 days (95% CI 2112–3555) (Olsen et al., 2007). The variability in estimating the human serum elimination half-life for PFBS must also be considered when the same male subject who participated in both the pilot and main studies had terminal serum elimination rates of 39.1 and 24.5 days, respectively. The only female subject had the highest serum elimination half-life of 45.7 days, nearly twice that of the geometric mean of the five male employees (24.1 days). The lack of additional female subjects, as well as the variation observed for the one male subject who was in the pilot and main studies, detracts from making any firm inference regarding sex-specific differences in the serum elimination half-life for humans.

We suspect that the marked difference in human PFBS elimination compared to PFHxS and PFOS may be due to the smaller molecular size of PFBS, its increased solubility, weaker and/or less extensive protein binding (Kerstner-Wood et al., 2003), or differences in affinity for anionic transport processes. The latter have been suggested to play a role in determining the pharmacokinetic properties of PFOS in monkeys (Andersen et al., 2006).

In modeling the serum PFBS elimination data, the best model fits were used. With the exception of the human serum PFBS elimination curves, this results in multi-phasic elimination profiles. Early time points were taken in conducting the kinetic studies with laboratory animals. This allowed profiling of early distribution phases. For humans, this was not possible, and the human kinetic profile appears to be based on one compartment.

The factors that result in the appearance of multiple compartments with PFBS in the laboratory animal studies have not been elucidated at this time. Kudo et al. (2002) initially speculated on a

possible role for the organic anion transporting polypeptide, oatp1, in reabsorption of the PFBS congener, perfluorooctanoate (PFOA), in the rat proximal tubule as a partial explanation for large sex differences in renal elimination of PFOA between male and female rats. Analysis of the kinetic data from single-dose i.v. studies with potassium perfluorooctanoate (K^+PFOA) and K^+PFOS in the same set of male and female cynomolgus monkeys (with the exception of one male) suggested a potential role for saturable renal tubular reabsorption (Andersen et al., 2006). In attempting to further understand the rat sex differences in elimination of PFOS, Kudo and colleagues have provided support for the involvement of oatp1 and OAT3 in the renal tubular transport, suggesting significant reabsorption of PFOA via oatp1 (Katakura et al., 2007). At this time, it is not known to what extent the kinetics of the perfluoroalkylsulfonates may be determined by organic anion transporter mediated processes and how these may differ between species and sex within species. Andersen et al. (2006) suggested that changing the transport maximum in a human pharmacokinetic model could account for the longer half-life of PFOS in humans as compared to monkeys.

Early elimination phases characterized by rapid elimination may represent, in part, saturation of reabsorption transport, if this is actually present, and, in part, redistribution from serum. Reported herein are estimates of the elimination rates for each apparent phase. Although these estimates may not represent the same active processes between species, they do allow comparison of elimination rates at serum concentrations that are quite small and in the range of those that approach expected values from potential exposure through the environment.

Human biomonitoring studies of the general population have not measured PFBS except for three studies. Calafat et al. (2007) detected PFBS in less than 0.4% of 2094 serum samples, collected in 2003–2004, of participants ≥12 years of age from the U.S. Centers for Disease Control and Prevention (CDC) National Health and Nutrition Examination Survey (NHANES) collected in 2003–2004. The limit of detection for PFBS was 0.4 ng/mL (0.0004 μg/mL). Olsen et al. (2008) analyzed PFBS in plasma samples collected in 2006 from 600 American Red Cross adult blood donors. A total of 593 plasma samples (98.8%) were reported below LOQ (0.3 ng/mL) (n = 480) or below LOQ (0.5 ng/mL) (n = 113). The remaining seven samples (1.2%) were measured above the LOQ with the highest concentration at 2.9 ng/mL. Likely explanations for the lack of quantifiable PFBS concentrations reported in these two studies are reduced production volume and the much shorter serum elimination half-life in humans, as shown in the present study, compared to that of PFOS. In the third study, Hölzer et al. (2008) reported 40,000 residents of Arnsberg, Germany were exposed by drinking water with perfluorochemical concentrations as a consequence of the widespread use of a soil conditioner which had been mixed with industrial waste. PFBS was reported above the limit of detection (0.1 ng/mL) in 33% of the blood samples of children, 4% of the women, and 13% of the men compared to 5%, 7%, and 3% of the reference (non-exposed) population, respectively. The referent population percentages are higher for PFBS than those reported by Calafat et al. (2007) likely due to the lower limit of detection used by Hölzer et al.

5. Conclusion

These studies have shown PFBS is more efficiently eliminated in rats, monkeys, and humans when compared to the six- and eight-carbon perfluorinated homologs, PFHxS and PFOS. Variations in the half-life of elimination of PFBS were observed across the three species with sex-specific elimination differences likely in the rat but not demonstrated in the monkey or human. These conclusions, however, are constrained by the limited number of subjects used in each of the studies reported herein. Collectively, these studies provide valuable insight for human health risk assessment regarding the potential for accumulation of body burden in humans on repeated exposure to PFBS-generating materials, whether from occupational, non-occupational (e.g., consumer), or environmental exposure sources of PFBS.

Conflict of interest

Geary Olsen, Shu-Ching Chang, David Ehresman, Paul Lieder, and John Butenhoff are employees of the 3M Company which manufactures PFBS and related materials. Patricia Noker and Gregory Gorman of Southern Research Institute were contracted by 3M to conduct some of the analyses in this study. The 3M Company funded this study.

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